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THE USE OF THYMIDINE IN STUDIES WITH MAMMALIAN CELL CULTURES

I. Clonal growth of HeLa S3 cultures after short incubations with tritium labeled thymidine

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by

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Some of the effects of tritium-labeled thymidine (H^3TdR) on the clonal growth of HeLa S3 cells have been reported (1 & 2). These studies quite clearly show that colony counts were depressed after incubation of the cells with small amounts of H^3TdR for 24 hours. However, results on the action of short incubations with H^3TdR were not so clear. Since H^3TdR is generally used for short incubations (so-called "pulse" or "flash" labeling) as a tracer for deoxyribonucleic acid (DNA) metabolism, it is of utmost importance to know if this treatment can cause cell death. Therefore the action of short incubations with H^3TdR on the clonal growth of HeLa S3 Cultures has been investigated more fully.

METHODS AND MATERIALS

The experimental procedures for single cell plating experiments were as outlined previously (1). HeLa S3 cells were inoculated into small petri dishes, including two into which cover slips had previously been placed, and after an 18 hour incubation to allow the cells to attach the medium was removed. Into one-third of the plates was added a medium containing 0.02 μc/ml H^3TdR at a specific activity of 5.1 c/mM; into another one-third was added a medium containing 0.02 μc/ml H^3TdR at 1.9 c/mM; into the other third was added a new medium containing no thymidine. The plates were placed in the incubator and after thirty minutes, three plates of each kind were removed and their medium discarded; the cells were washed twice with Hanks' balanced salt solution (HBSS), a medium containing 20 μg/ml unlabeled thymidine added, and

the plates were then replaced in the incubator. Other plates were treated similarly at 6 hours and 24 hours after the addition of H^3TdR. The cells then incubated for 14 days, at which time all media were removed and the colonies that had developed were counted, after being fixed with acetic alcohol (1:3) and stained with Giemsa.

The cells on the two plates with cover slips (one with 5.1 c/mM medium, the other with 1.9 c/mM medium) were fixed immediately after the tracer incubation. The cover slips were removed, mounted on 1 x 3 inch microscope slides and covered with stripping film. The resulting autoradiograms were exposed for two weeks, developed, stained with Giemsa, and examined for the presence of grains resulting from tritium-labeled nuclei.

In the second experiment the specific activity was kept constant (1.9 c/mM) but varying amounts of H^3TdR (0.02, 0.1, 0.5 and 2.5 μc/ml) were incubated with the cells. One-half of the cultures at each level of isotope were incubated for 30 minutes, the other one-half for 24 hours, before removal of the radioisotope, washing and reincubation with the medium containing 20 μg/ml carrier thymidine. In this experiment, four plates that were never exposed to any thymidine were also included.

RESULTS

The effects of 0.02 μc/ml H^3TdR (two levels of specific activity) are shown in Table I. It is quite evident that this amount of H^3TdR has no effect on the viability of HeLa S3 cells when incubation is limited to thirty minutes. Autoradiograms prepared from the cover slip

cultures showed that, after the thirty minutes incubation with H^3TdR, 29 per cent of the cells were labeled. This is in line with our previous data (3) and shows that uptake occurred to an extent that can be used for autoradiographic experiments.

In the second experiment (Table II) an unusually low number of colonies appeared in control plates. Regardless of whether this resulted from an unaccountable low plating efficiency or from a dilution or counting error, the results unequivocally show that the incorporation of H^3TdR in the medium for thirty minutes, at all levels tested, had no effect on ultimate survival. The effect of 0.02 μc/ml for 24 hours is again in doubt, but at all other concentrations of H^3TdR, the 24 hour incubation resulted in a complete absence of colonies.

The four plates that were never exposed to any thymidine yielded about 65 per cent more colonies than control plates which had been exposed to 20 μg/ml unlabeled thymidine throughout the experiment. If the former cultures are considered the controls for unlabeled thymidine effects, it appears that 20 μg/ml thymidine depresses colony formation by about 40 per cent. In light of other experiments, reported in the accompanying communications (4) the effect may have been exaggerated somewhat by the effect that caused the general low colony formation in this experiment. Nevertheless, it does indicate that long incubations with thymidine can result in cell death.

DISCUSSION

The results presented here show that H^3TdR can be safely used as

a tracer for DNA metabolism in mammalian cell cultures, when its incubation is limited to one-half hour. The failure of even relatively high concentrations (2.5 μc/ml) with thirty minute incubations to cause a depression in colony counts confirms our previous observations (2) and emphasizes that the lethal effect of H^3TdR is dependent on time of incubation as well as concentration. This evidence along with our previous data showing that tracer amounts do not affect the generation time or mitotic index in the first two generations following its administration (3) demonstrate unequivocally that H^3TdR can be used in pulse labeling experiments with no danger of disturbing normal metabolism or growth. On the other hand, the effect of unlabeled thymidine in the medium for long periods of time does appear to cause disturbing results. The results of further experiments on this point are discussed thoroughly in the accompanying report (4).

SUMMARY

Thirty minute incubations with tritiated thymidine, at concentrations from 0.02 to 2.5 μc/ml, and at two specific activities, did not affect the production of colonies of HeLa S3 cultures, but longer incubations did cause a depression in number of colonies.

REFERENCES

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TABLE I

THE EFFECTS OF 0.02 μc/ml TRITIUM-LABELED THYMIDINE, AT TWO SPECIFIC ACTIVITIES, ON THE CLONAL GROWTH OF HELA S3 CELLS

Hours of incubation with tracer	Number of Colonies developed		
	Control	0.02 μc H^3TdR/ml 1.9 c/m^M	0.02 μc H^3TdR/ml 5.1 c/m^M
1/2	167 ± 12	167 ± 10	160 ± 3
6	150 ± 8	137 ± 10	138 ± 0
24	164 ± 4	68 ± 39*	68 ± 31*

*High variability due to 1 plate having counts near the mean of control, while other 2 plates had low counts.

TABLE II

CLONAL GROWTH OF HELA S3 CELLS AFTER INCUBATION FOR ONE-HALF OR 24-HOURS IN THE PRESENCE OF VARYING AMOUNTS OF TRITIUM LABELED THYMIDINE

Hours of incubation with tracer	Number of Colonies developed				
	Control	0.02 μc/ml	0.1 μc/ml	0.5 μc/ml	2.50 μc/ml
1/2	44 ± 5	40 ± 4	45 ± 2	43 ± 3	44 ± 4
24	46 ± 3	38 ± 8	0	0	0
0*	75 ± 7				

* Four plates that received no thymidine of any kind, including the 20 μg/ml carrier that all other plates contained.